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Priapism Following Splenectomy in an Unstable Hemoglobin: Hemoglobin Olmsted β141 (H19) Leu→Arg

Isabelle Thuret, Josiane Bardakdjian, Catherine Badens, Henri Wajcman, Frederic Galacteros, Danielle Vanuxem, Henri Perrimond, Francis Giraud, and Danielle Lena-Russo

Hématologie Pédiatrique (I.T., H.P.) et Centre d'Enseignement et de Recherche in Génétique Médicale (C.B., F.G., D.L.-R.) CHU-Timone, Marseille, France, + INSERM U 91, Hôpital Henri Mondor, Créteil, France (J.B., H.W., F.Ga.), Explorations fonctionnelles respiratoires, Hôpital Sainte-Marguerite, Marseille, France (D.V.)

We report a case of severe priapism occurring in a patient with an unstable hemoglobin, Hb Olmsted (β141 Leu→Arg). This is a rare hemoglobin variant, which until now has been reported only once. The clinical course of the 12-year-old boy was characterized by severe hemolytic anemia leading to splenectomy and cholecystectomy at the age of 3.5 years. The priapism occurred 8 years after splenectomy, during a hemolytic febrile episode and required aspiration of the corpora cavernosa. This report raises the question of the benefit of splenectomy in patients suffering from a chronic hemolytic anemia such as that due to an unstable hemoglobin. This treatment lowers the frequency and the severity of acute hemolytic attacks, but several cases of vascular complications have been reported after splenectomy. © 1996 Wiley-Liss, Inc.

Key words: unstable hemoglobin, priapism, splenectomy

INTRODUCTION

The clinical expression of a highly unstable hemoglobin variant is a severe hemolytic anemia. This anemia is partly due to an excessive trapping, by an enlarged spleen, of red blood cells (RBC) that contain Heinz bodies and have membrane alterations, with decreased plasticity. Many anemic patients carrying an unstable hemoglobin have undergone splenectomy to reduce hypersplenism and to improve the hemoglobin level. After splenectomy, thromboembolic complications have been reported in several instances.

Hemoglobin Olmsted, which is characterized by the replacement of the leucine residue at position β141 (H19) by an arginine, is among the more clinically severe of the unstable hemoglobins. In addition to the case reported here, it has been observed in only one other patient, who also suffered from a severe hemolytic anemia. This patient was clinically described in 1969 by Fairbanks et al. [1] and the structural abnormality of the hemoglobin was identified in 1975 by Lorkin et al. [2].

In this paper we describe the clinical course of a boy, 12 years old. In his first years of life he was observed to have a chronic hemolytic anemia of unidentified origin. Following splenectomy, an abnormal hemoglobin fraction appeared on electrophoresis and Heinz bodies were found in the peripheral blood, indicating the presence of an unstable hemoglobin, further characterized as hemoglobin Olmsted. A severe episode of priapism was observed 8 years after splenectomy.

MATERIALS AND METHODS

Hematological Studies

Hematological data were obtained with an automatic cell counter and with routine hematological procedures. For Heinz bodies count, RBC were incubated at 37°C and stained with methyl violet [3] and with cresyl blue. Hemolysates were studied by electrophoretic analysis on cellulose acetate at alcaline pH, citrate agar, isoelectric focusing (IEF), and globin chain electrophoresis in urea, pH 6 and 9. Quantification of fetal hemoglobin was done by the alkali denaturation method and ion-exchange chro-

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Address reprint requests to Dr. I. Thuret, Service d'Hématologie Pédiatrique, Hôpital d'Enfants "La Timone," Bd J. Moulin, 13385 Marseille, France.

matography. The stability of hemoglobin was studied with the isopropanol test.

Hemoglobin Studies

Structural identification was achieved as previously described [4]. The peptides of the abnormal β-chain were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) and analyzed for their amino acid composition. To confirm the mutation, a 404 bp DNA fragment spanning the region of codon 141 was amplified from genomic DNA by polymerase chain reaction (PCR) using primers E1 and F1 [5]. The PCR product was further digested with *Msp*I restriction endonuclease and analyzed by electrophoresis.

Globin chain biosynthesis was performed as described by Leone et al [6]. Red blood cells were incubated for 2 hr at 37°C with L-^{3}H-leucine. The α- and β-globin chains were separated by RP-HPLC and collected.

RESULTS

Case Report

The patient was born in 1981 from a French mother and a Spanish father. He developed a prolonged neonatal jaundice and hepatosplenomegaly, and at the ages of 2 and 4 months he had two severe anemic episodes requiring blood transfusions. At 8 months, the RBC parameters revealed a slightly hypochromic anemia (Hb 8.5 g/dl, MCH 25.5 pg), with mild macrocytosis (MCV 92 fl). Basophilic stipplings, normoblastemia (3 nucleated RBC/100 WBC), and a few spherocytes were observed on the blood smears. The reticulocyte count was increased to 436 × 10^{9}/liter (14%). Bilirubin level was 82 μmol/liter. Electrophoresis on cellulose acetate and citrate agar as well as IEF failed to reveal a hemoglobin variant. The hematological parameters and electrophoretic pattern of the other members of the family (mother, father, and brother) were normal.

The clinical course was characterized by a chronic anemia, with hemoglobin values between 7 and 8 g/dl. A progressive enlargement of the spleen and, at the age of 3.5 years, the occurrence of gallstones were observed. A study of the ^{59}Fe distribution showed the presence of a severe peripheric hemolytic process and a massive RBC sequestration by the spleen. A splenectomy and, at the same time, a cholecystectomy were performed in June, 1985. The hematological indices following splenectomy are shown in Table I. The hemoglobin value increased to 9 g/dl, but a high level of hemoglobin F persisted (10–13%). Platelet, nucleated RBC (NRBC), and reticulocyte counts increased markedly. Macrocytosis, anisocytosis, polychromatophilia, and cells containing Howell-Jolly bodies or basophilic stipplings were observed in the blood smears. The electrophoretic study of the hemolysate then revealed the presence of several abnormal, slow-moving hemoglobin components. They amounted to approximately 5% of the total hemoglobin. The isopropanol test was slightly and irregularly positive.

The patient, at the age of 12 years, was readmitted in May, 1993, for pallor, dark urine, and abdominal pain following a febrile episode. Hemoglobin value was 4.5 g/dl, and a transfusion was given. Two days after admission, he developed a prolonged priapism requiring aspiration of the corpora cavernosa. The platelet count was 819 × 10^{9}/liter and nucleated RBC 12 × 10^{9}/liter. The patient's activated partial thromboplastin time was prolonged at twofold the normal value and was not corrected by mixture with equal volume of normal plasma. Anticardiolipin antibodies (ACA, IgG ELISA) were found to be elevated (53 Harris units). Following surgery, the priapism resolved, and anticoagulant as well as ACA disappeared within 2 months. Protein C antigen, protein C activity (chromogenic and chronometric determination), and protein S activity, measured 3 months after the thrombotic event, were normal. Priapism did not recur over the following year.

Hemoglobin Characterization

The HPLC elution profile of the tryptic digest of the mutated β-chain demonstrated the absence of peptide β-T14 and the presence of an abnormal peak eluting before peptide β-T1. Its amino acid composition revealed that it resulted from the replacement of the leucine residue at position 141 by an arginine, leading to a tryptic cleavage at this position. This variant was therefore identified to hemoglobin Olmsted (β141 [H19] Leu→Arg).

The replacement of the second nucleotide of codon 141, which is responsible for the amino acid change of hemoglobin Olmsted, leads to a restriction site for endonuclease *Msp*I (C*T*G G→C*C*G G). A 404 bp DNA fragment spanning the region of codon 141 was amplified via PCR from the genomic DNA of the patient. After digestion with *Msp*I, the predicted hemoglobin Olmsted-specific fragments of 193 and 211 bp were observed. Because of the high HbF level, the −158 C→T mutation on the propositus Gγ gene was investigated. It was absent on both chromosomes.

Biosynthetic Studies

Separation of in vitro-labeled globin chains showed an abnormal peak eluting before the normal β^{A} chain (Fig. 1). The α/non-α ratio of incorporated radioactivity was equilibrated after 2 hr of incubation. The β-chain of Hb Olmsted accounted for 32%, and the γ-chains 13%, of the radioactivity incorporated in the non-α-chains. The specific activity of the β-chain of Hb Olmsted was three times that of the normal β-chain, indicating a rapid post-translational turnover of Hb Olmsted.

DISCUSSION

This second report of a patient carrying Hb Olmsted shares many features with the previously reported case.

TABLE I. Hematological Data Before (1981–1984) and After (1985–1993) Splenectomy

	1981	1982	1983	1984	1985	1990	1993
Hb (g/dl)	8.0	7.5	7.2	6.8	8.8	8.9	9.6
MCV (fl)	92	83	84	82	83	108	109
MCH (pg)	25.5	24.6	27.1	25.8	29	34	32.9
MCHC (%)	27.6	29	31.9	31.5	35	30.9	31
Reticulocytes (%)	10				30	32	45
NRBC (10^9/liter)	0.2				18	10.5	11
HbF (%)		19	25	35	10	13	10
Platelets (10^9/liter)	200–400				939	904	850
Bilirubin (μmol)	62	50	73	52	106	49	72

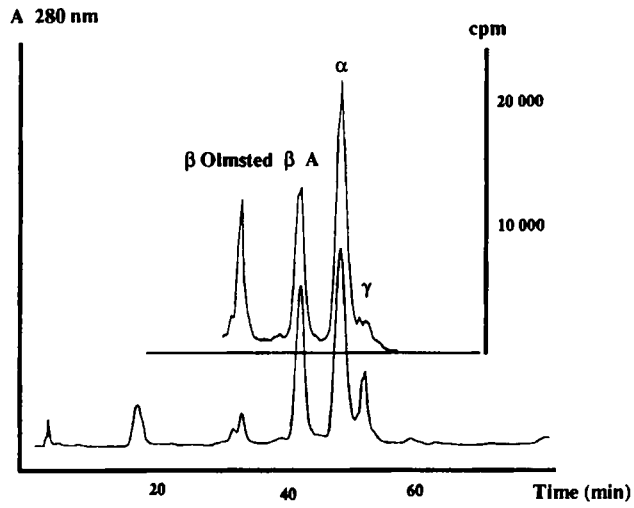

Fig. 1. Separation of in vitro-labeled globin chains. Experimental conditions: Merck LiChrospher 100 ch-8/2 (250 × 4mm). The β Olmsted chain, eluted at 33 min, displays a high specific activity relative to the normal β-chain.

Both of the patients showed de novo mutations and both patients had suffered since infancy from a severe anemia and were treated by splenectomy at an early age. Anemia improved following splenectomy: Less frequent blood transfusions were required in the case described by Fairbanks et al. and an increase in the Hb value was noticed in our patient. In the two patients the Hb F level was high, and no inclusion bodies were found before splenectomy. The high level of Hb F in our patient does not correlate with the presence of the *Xmn*I site or hereditary persistence of fetal hemoglobin, the Hb F in both parents being less than 1%. Hb Olmsted, because of its high instability and its low proportion, could be detected only by electrophoresis after splenectomy in our patient. These properties also explain why the isopropanol tests were negative or questionable.

The original patient with Hb Olmsted died of a chronic pulmonary disease with pulmonary hypertension at age 36 years [7]. In our case, a priapism occurred 8 years after splenectomy. Several mechanisms probably contributed to priapism.

First, priapism following splenectomy has been reported in patients with or without underlying hematological disease. Recurrent episodes of priapism in the first weeks or months postsplenectomy have been described in 3 young adults with α- or β-thalassemia intermedia [8–10]. Priapism has also been reported several years after surgery in three adult patients splenectomized for abdominal trauma [11]. Thrombocytosis and elevated nucleated RBC count are usually considered as the two major hyperviscosity factors. In our report, platelet count and nucleated RBC were markedly increased. Moreover, in cases of unstable hemoglobin, splenectomy probably increases RBC rigidity and adherence to endothelial cells by inducing reticulocytosis and cells containing Heinz or Howell-Jolly bodies. Priapism and pulmonary hypertension are well-known complications of sickle cell disease (SCD). Many intrinsic abnormalities of SCD RBC have been implicated as causes of rigidity and increased adhesiveness: membrane sialic acid abnormalities, oxidative membrane damage, loss of membrane phospholipid asymmetry, and binding to various plasma adhesive proteins (fibrinogen, fibronectin, thrombospondin, von Willebrand factor) [12–15]. Some of these factors may also be present in unstable hemoglobins [16]. Second, priapism followed an acute viral illness, with transient anticardiolipin antibodies. Transient antiphospholipid antibodies during febrile episodes in children are usually not associated with thromboocclusive tendency [17]. Nonetheless their precipitating role cannot be excluded in this context. Third, the onset of puberty in this 12-year-old boy may be regarded as a contributive factor.

Other cases of thromboocclusive complications have been reported in unstable hemoglobins. A 32-year-old patient with hemoglobin Hradec Kralove experienced frequent thrombophlebitic complications in the lower extremities [18]. Pulmonary embolism or pulmonary hypertension has been reported in hemoglobins Köln, Duarte, Santa Ana, and Warsaw [19–22]. In our own experience, right atrium thrombosis and visceral infarctions were the respective causes of death of two patients with Hb Ham-

mersmith and Hb Bicêtre. All these patients had been splenectomized, and most of them had a severe form of unstable hemoglobin. In spite of the numerous potential mechanisms that might contribute to priapism, the present report raises the question of the vascular risk of splenectomy in those with severe unstable hemoglobin.

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